Journal of Medical Virology 53:185–188 (1997)

Human Papillomavirus Type 11 Neutralization in the Athymic Mouse Xenograft System: Correlation With Virus-Like Particle IgG Concentration

Janine T. Bryan,[3] Kathrin U. Jansen,[5] Robert S. Lowe,[5] Kenneth H. Fife,[1,3,4] Tracy McClowry,[1] Dessa Glass,[1] and Darron R. Brown[1,2,3]*

[1]*Department of Medicine, Division of Infectious Diseases, Indiana University School of Medicine, Indianapolis, Indiana*
[2]*Richard L. Roudebush Veterans Administration Medical Center, Indiana University School of Medicine, Indianapolis, Indiana*
[3]*Department of Microbiology and Immunology, Indiana University School of Medicine, Indianapolis, Indiana*
[4]*Department of Pathology, Indiana University School of Medicine, Indianapolis, Indiana*
[5]*Merck Research Laboratories, West Point, Pennsylvania*

Neutralization of virus is likely to be necessary for development of an effective prophylactic vaccine against genital human papillomavirus (HPV) infection. Two New Zealand white rabbits were immunized with purified HPV type 11 (HPV 11) virions in Freund's adjuvant. An enzyme linked immunoassay (ELISA) was used to determine the quantity of IgG which recognized the HPV 11 major capsid protein (L1 protein) virus-like particles (VLPs) in the two anti-HPV 11 sera (serum A and serum B). The concentration of HPV 11 L1 VLP-specific IgG in the A and B sera were determined to be 37 and 90 µg per ml, respectively. The A and B sera were used in neutralization experiments in the athymic mouse xenograft system with known quantities of purified HPV 11 virions. The concentration of HPV 11 L1 VLP-specific IgG required to neutralize HPV 11 was determined for each antiserum. This concentration of IgG was approximately 700 to 900 ng per ml. This study demonstrates a positive correlation between the level of HPV 11 L1 VLP-specific IgG in animals immunized with HPV 11 virions and neutralization of HPV 11 in the athymic mouse model. Further studies are needed 1) to determine if sera or genital secretions from other species are neutralizing in the athymic mouse xenograft system, and 2) to determine if the VLP ELISA can be used as a reliable substitute for more cumbersome neutralization assays. ***J. Med. Virol. 53:185–188, 1997.***

KEY WORDS: human papilloma-virus; antibody neutralization; athymic mice

INTRODUCTION

Antiserum or monoclonal antibodies raised against human papillomavirus type 11 (HPV 11) virions or virus-like particles (VLPs) have been shown to neutralize HPV 11 infection of human foreskin implants grown in athymic mice [Bonnez et al., 1992; Christensen et al., 1990, 1992, 1994; Christensen and Kreider, 1990]. Because HPVs cannot be grown by standard culture methods, the mouse xenograft system provides an important model for testing the ability of antibodies to protect against HPV infection [Kreider et al., 1986, 1987]. It is not known if HPV 11 neutralization in the athymic mouse system correlates with the amount of specific antibodies in serum or other antibody-containing fluids. To determine if neutralization of HPV 11 in the mouse xenograft system correlates with antibodies to native virus, several neutralization experiments were performed using one of two polyclonal rabbit sera raised against purified HPV 11 virions. The concentration of the HPV 11 major capsid protein (L1 protein) VLP-specific IgG in the anti-HPV 11 sera was established by ELISA. Anti-HPV 11 sera were used in neutralization experiments in the mouse xenograft system, using known, standardized quantities of purified HPV 11 virions. The concentration of HPV 11 L1 VLP-specific IgG for each serum was then correlated with the neutralizing titer of the sera.

MATERIALS AND METHODS

Production of Anti-HPV 11 Sera

HPV 11 virions were purified by cesium gradient centrifugation from HPV 11-infected foreskin tissue

*Correspondence to: Darron R. Brown, Department of Medicine, Division of Infectious Diseases, Indiana University School of Medicine, Indianapolis, IN.

Accepted 5 May 1997

grown in athymic mice as described previously [Brown et al., 1994]. The viral protein content of the HPV 11 preparation was determined to be 1 ng/μl by first ascertaining the total protein content of the preparation (Pierce, BCA Protein Assay) and then estimating the percentage of the major capsid (L1) protein in the preparation by Coomassie stained SDS-PAGE gels and immunoblots using anti-L1 serum [Brown et al., 1994]. To prepare polyclonal antisera, two New Zealand white rabbits were immunized with 10 ng of non-denatured virions emulsified in complete Freund's adjuvant, followed by four booster immunizations (10 ng each) given at 2-week intervals, in incomplete Freund's adjuvant. Sera from the two rabbits was collected 9 weeks after the initial immunization and given the designations anti-HPV 11 A and anti-HPV 11 B.

Enzyme-Linked Immunosorbent Assay (ELISA)

The level of HPV 11 VLP IgG antibody in undiluted rabbit anti-HPV 11 A and B serum was determined by capture ELISA. Monoclonal antibody H11 B2 specific for HPV 11 VLPs (Chemicon) was bound to the ELISA wells. The wells were then washed with TTBSM (20 mM Tris, 137 mM NaCl, 0.1% Tween 20, pH 7.6) and blocked with TTBSM containing 10% milk. HPV 11 L1 VLPs derived from recombinant baculoviruses [Christensen et al., 1994] were added to the wells (5–10 ng/well) for 1 hour. Wells were then washed and dilutions of the anti-HPV 11 test sera were added. After a 1 hour incubation, wells were washed and bound anti-HPV 11 VLP immunoglobulin (VLP-specific IgG) was detected by a 1:1,000 dilution of goat anti-rabbit IgG (Pierce). HPV 11 L1 VLP-specific IgG concentrations were determined by endpoint dilution and quantified against a standard curve generated with purified rabbit IgG standards (Pierce).

Neutralization Assays

Foreskin tissue was obtained from routine circumcisions. Dermis was removed from the foreskin tissue, and the remaining epidermal tissue was cut into 1 × 1 mm fragments. Four foreskin tissue fragments sufficient for implantation into two mice were added to a tube containing Minimal Essential Medium (MEM) (Sigma), 40 μl of purified HPV 11 virions (1 ng/μl of viral protein, corresponding to approximately 10^7 virions per microliter), and test serum diluted in MEM. The final volume of each incubation was 200 μl.

The foreskin fragments, purified HPV 11 virions, and test serum were combined simultaneously and incubated at 37°C in a heat block for 90 minutes. Foreskin fragments were then implanted under the renal capsules of athymic mice. Preimmune or anti-HPV 11 A or B sera were tested at several dilutions (1:25, 1:50, 1:100, 1:250, 1:500, 1:1,000, 1:2,000, or 1:3,000) in the neutralization assay, using a minimum of four tissue fragments for each dilution. Our previous studies have shown that HPV DNA is detected in HPV 11-infected human foreskin implants by in situ hybridization 6 weeks after implantation, and histologic changes characteristic of HPV infection are present 6 to 8 weeks after implantation [Brown et al., 1995]. To ensure HPV detection, mice were killed 10 weeks after tissue implantation. Implants were removed from the mice, measured in three dimensions as described by Bonnez et al. [1992], and placed in a formalin solution for preparation of paraffin embedded sections.

DNA In Situ Assay

Sections of foreskin implants were deparaffinized and processed with the Digene Tissue Hybridization Kit (Digene Diagnostics, Beltsville, MD) as recommended by the manufacturer. Sections from each tissue were deparaffinized with xylene and ethanol, heated for 5 minutes at 100°C, then hybridized for 18 hours at 37°C using a biotinylated genomic HPV 11 DNA probe. Detection of hybridized probe was performed by incubation of slides with a streptavidin-alkaline phosphatase conjugate and reaction with 5-bromo-4-chloro-3-indolyl phosphate/nitroblue tetrazolium substrate. Cells positive for HPV DNA were identified as purple nuclear staining of differentiated epithelial cells in the foreskin implants by light microscopy. One section from each implant was stained with hematoxylin and eosin for confirmation of histology consistent with HPV infection.

RESULTS

Characterization of Anti-HPV 11 Serum by ELISA

The concentration of HPV 11 L1 VLP-specific IgG in the anti-HPV 11 A serum was determined by ELISA to be 37 μg/ml (data not shown). The VLP-specific IgG concentration in the B serum was 90 μg/ml.

Neutralization Assays

Neutralization of HPV 11 was demonstrated by an absence of HPV DNA in a DNA in situ hybridization assay (Fig. 1). HPV DNA-positivity roughly correlated with implant size, but there were several small implants with strong positive signals for HPV DNA. Implants containing only subtle epithelial abnormalities suggesting HPV infection, such as epithelial thickening or nuclear atypia, were consistently HPV DNA positive. Implants with epithelial abnormalities strongly suggestive of HPV infection, such as the presence of koilocytes, were always positive for HPV DNA in the in situ assay.

Preimmune serum diluted 1:50 had no ability to neutralize HPV 11, as greater than 85% of implants (combined from several assays) were positive for HPV DNA in the in situ hybridization assay. This proportion of HPV-positive implants was nearly identical to that seen with no added serum (data not shown). For the anti-HPV 11 sera, a dilution of 1:50 for anti-HPV 11 serum A (740 ng/ml) and 1:100 for anti-HPV 11 serum B (900 ng/ml) effectively neutralized HPV 11 infection, with less than 10% of implants being positive for HPV DNA (Table I).

Plotting HPV 11 VLP-specific IgG concentrations vs.

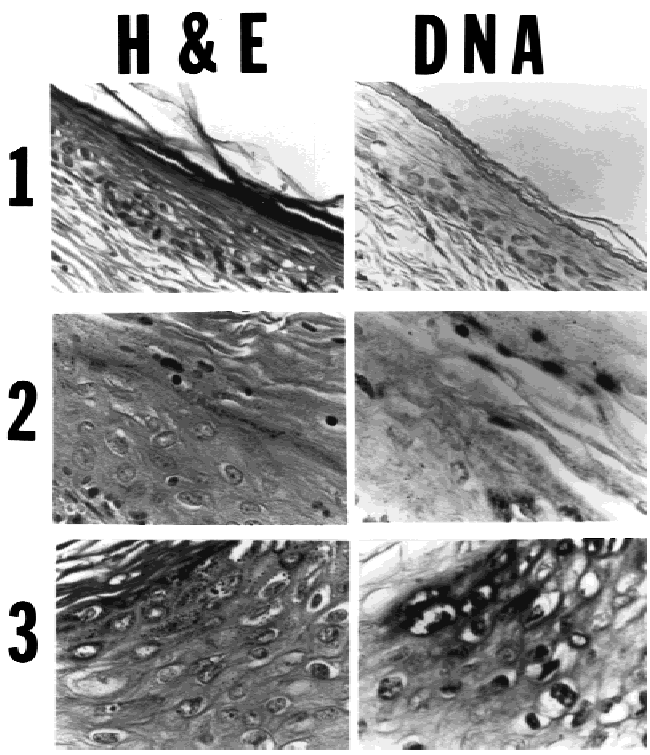

Fig. 1. Histology and HPV DNA in situ hybridization assay of three HPV 11-infected human foreskin implants grown in athymic mice. All were part of the neutralization experiments described in the text, and are shown to illustrate tissue of an effective neutralization (number 1 on the left side of the illustration), and two implants that contain HPV DNA (numbers 2 and 3), and are therefore not neutralized by the antiserum. The panel labelled H & E contains sections of implants stained with hematoxylin and eosin; the panel labelled DNA contains sections of the same implants analyzed for HPV DNA by in situ hybridization. Implant number 1 contains histologically normal human epithelium, and was negative in the HPV DNA in situ assay. Anti-HPV 11 serum with a VLP-specific IgG concentration of 370 ng/ml was utilized in this neutralization assay. Implant number 2 contains moderately thickened epithelium and enlarged nuclei in the differentiated cells, which are at the top of the figure. The most differentiated cells are positive (darkly stained nuclei in the photographs, which are bright purple in color) in the HPV DNA in situ assay. Anti-HPV 11 serum with a VLP-specific IgG concentration of 19 ng/ml was used in this neutralization assay. Implant number 3 contains markedly thickened epithelium and koilocytes. Numerous nuclei are positive for HPV DNA. Anti-HPV 11 serum with a VLP-specific IgG concentration of 19 ng/ml was used in this neutralization assay. Original magnification 40×.

percent neutralization of HPV 11 infection for each serum produced a curve from which the estimated concentration of HPV 11 VLP-specific IgG capable of completely neutralizing HPV 11 infection was derived (not shown). The estimated neutralizing concentration of HPV 11 L1 VLP-specific IgG was determined to be approximately 700 ng per ml for both anti-HPV 11 A and anti-HPV 11 B sera. As mentioned above, complete neutralization of HPV 11 was demonstrated with a serum dilution containing a VLP-specific IgG concentration in this range (Table I).

TABLE I. Comparison of the Anti-HPV 11 A and B Sera

	VLP-specific IgG concentration	HPV 11 neutralization	
anti-HPV 11 A	19	0/8	0%
	74	3/12	25%
	370	6/8	75%
	740	16/16	100%
anti-HPV 11 B	30	0/4	0%
	45	1/4	25%
	90	1/4	25%
	180	8/8	100%
	360	7/8	88%
	900	8/8	100%
	1800	8/8	100%
	3600	8/8	100%

Neutralization data is shown for each VLP-specific IgG concentration (ng/ml) used. Neutralization data is expressed as a ratio of the number of HPV 11 DNA-negative implants over the number of implants recovered. This ratio is shown as a percentile in the next column.

DISCUSSION

This study suggests that a measure of the HPV 11 L1 VLP-specific IgG concentration can be correlated with the neutralization titer for two different polyclonal rabbit sera raised against HPV 11 virions. In addition, by applying the VLP-specific IgG concentration and neutralization titer correlation, one can predict that a serum sample containing greater than approximately 700 to 900 ng per ml of IgG reactive against HPV 11 L1 VLPs should effectively neutralize HPV 11 virus in the mouse xenograft system using the conditions described in this study.

A number of techniques have been described to demonstrate neutralization of HPV 11 [Bonnez et al., 1992; Christensen et al., 1990, 1992, 1994; Christensen and Kreider, 1990]. We required the absence of HPV DNA by in situ hybridization as evidence of neutralization. The DNA in situ hybridization assay was used because it has a defined sensitivity and is a reproducible assay. Histologic evaluation of implants alone was inadequate to verify neutralization as several implants were histologically normal or minimally abnormal, but contained HPV DNA in the nuclei of differentiated epithelial cells as determined by the in situ assay. In addition, while differences in the size of the implants, measured in mean geometric diameter, were roughly correlated with HPV DNA positivity, size alone was inadequate to confirm neutralization. Several very small implants were clearly positive for HPV DNA (data not shown). We feel that DNA in situ hybridization is a more rigorous and representative measure of HPV infection.

If an HPV 11 L1 VLP-specific IgG concentration of approximately 700 to 900 ng per ml is achievable in cervicovaginal secretions, it is possible that the cervix may be protected against HPV 11 infection. This assumes that neutralization in the mouse xenograft system mimics natural cervical infection conditions. It is possible that less antibody may be required to protect the cervical mucosa from natural infection than was needed in the athymic mouse xenograft system described, because the large quantity of purified, concentrated virions used in our experiments (approximately 10^8 virions per implant) may exceed the amount of vi-

rus encountered in a natural infection. For reproducibility of experiments, we chose to use a large quantity of virions for these studies, which consistently caused at least 80% infectivity in previous experiments (unpublished data). Further studies will be needed to determine if the presence of antibodies in cervicovaginal secretions are capable of protection against HPV infection, either in the mouse xenograft system or against natural HPV infections.

In summary, polyclonal rabbit serum raised against purified HPV 11 virions were produced and tested for the antibodies against HPV 11 L1 VLPs and for the ability to neutralize HPV 11 infection in the athymic mouse xenograft system. A good correlation between the HPV 11 L1 VLP-specific IgG concentration and the HPV 11 neutralizing titer was found for the two rabbit sera raised against purified HPV 11 virions. This study suggests that it may be possible to predict the neutralizing ability of a serum or secretion and possibly predict protection against natural HPV 11 infection based on the VLP-specific IgG concentration as determined by ELISA. The ELISA format would be much easier to apply to larger numbers of subjects. Previous neutralization assays using the athymic mouse xenograft system have not used known quantities of virus or sera of known specific immunoglobulin concentration. In addition, most have used implant size or histology as evidence of HPV infection. The VLP-specific IgG concentration of antisera in prior reports was not measured or correlated with HPV 11 neutralization. The neutralization assay described above provides a standardized method of quantifying antibody-mediated neutralization of HPV 11 in the mouse xenograft system, permitting comparisons of neutralization data between laboratories working with this system in research and vaccine development.

REFERENCES

Bonnez W, Rose RC, Reichman RC (1992): Antibody-mediated neutralization of human papillomavirus type 11 (HPV 11) infection in the nude mouse: Detection of HPV-11 mRNAs. Journal of Infectious Diseases 165:376–380.

Brown DR, Fan L, Jones J, Bryan J (1994): Colocalization of human papillomavirus type 11 E1^E4 and L1 proteins in human foreskin implants grown in athymic mice. Virology 201:46–54.

Brown DR, Bryan JT, Pratt L, Handy V, Fife KH, Stoler MH (1995): Human papillomavirus type 11 E1^E4 and L1 proteins colocalize in the mouse xenograft system at multiple time points. Virology 214:259–263.

Christensen ND, Kreider W (1990): Antibody-mediated neutralization in vivo of infectious papillomaviruses. Journal of Virology 64: 3151–3156.

Christensen ND, Kreider JW, Cladel NM, Patrick SD, Welsh PA. (1990): Monoclonal antibody-mediated neutralization of infectious human papillomavirus type 11. Journal of Virology 64:5678–5681.

Christensen ND, Kreider JW, Shah KV, Rando RF (1992): Detection of human serum antibodies that neutralize infectious human papillomavirus type 11 virions. Journal of General Virology 73:1261–1267.

Christensen ND, Höpfl R, DiAngelo SL, Cladel NM, Patrick SD, Welsh PA, Budgeon LR, Reed CA, Kreider JW (1994): Assembled baculovirus-expressed human papillomavirus type 11 L1 capsid protein virus-like particles are recognized by neutralizing monoclonal antibodies and induce high titres of neutralizing antibodies. Journal of General Virology 75:2271–2276.

Kreider JW, Howlett MK, Lill NL, Bartlett GL, Zaino RJ, Sedlacek TV, and Mortel R (1986): In vivo transformation of human skin with human papillomavirus type 11 from condylomata acuminata. Journal of Virology 59:369–376.

Kreider JW, Howett MK, Leure-Dupree AE, Zaino RJ, Weber JA (1987): Laboratory production in vivo of infectious human papillomavirus type 11. Journal of Virology 61:590–593.